

PACAP receptor gene polymorphism impacts fear responses in the amygdala and hippocampus

Jennifer Strafford Stevens^{a,1}, Lynn M. Almlie^a, Negar Fani^a, David A. Gutman^b, Bekh Bradley^{c,a}, Seth D. Norrholm^{c,a}, Emily Reiser^a, Timothy D. Ely^a, Rahim Dhanani^a, Ebony M. Glover^a, Tanja Jovanovic^a, and Kerry J. Ressler^{a,d}

^aDepartment of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA 30322; ^bDepartment of Biomedical Informatics, Emory University School of Medicine, Atlanta, GA 30322; ^cMental Health Service Line, Atlanta Veterans Affairs Medical Center, Decatur, GA 30033; and ^dHoward Hughes Medical Institute, Chevy Chase, MD 20815

Edited by Bruce S. McEwen, The Rockefeller University, New York, NY, and approved January 9, 2014 (received for review October 9, 2013)

We have recently found higher circulating levels of pituitary adenylate cyclase-activating polypeptide (PACAP) associated with posttraumatic stress disorder (PTSD) symptoms in a highly traumatized cohort of women but not men. Furthermore, a single nucleotide polymorphism in the PACAP receptor gene *ADCYAP1R1*, adenylate cyclase activating polypeptide 1 receptor type 1, was associated with individual differences in PTSD symptoms and psychophysiological markers of fear and anxiety. The current study outlines an investigation of individual differences in brain function associated with *ADCYAP1R1* genotype. Forty-nine women who had experienced moderate to high levels of lifetime trauma participated in a functional MRI task involving passive viewing of threatening and neutral face stimuli. Analyses focused on the amygdala and hippocampus, regions that play central roles in the pathophysiology of PTSD and are known to have high densities of PACAP receptors. The risk genotype was associated with increased reactivity of the amygdala and hippocampus to threat stimuli and decreased functional connectivity between the amygdala and hippocampus. The findings indicate that the PACAP system modulates medial temporal lobe function in humans. Individual differences in *ADCYAP1R1* genotype may contribute to dysregulated fear circuitry known to play a central role in PTSD and other anxiety disorders.

fMRI | emotion | intermediate phenotype | single nucleotide polymorphism

Posttraumatic stress disorder (PTSD) is an anxiety disorder estimated to affect 7% of the population (1), with symptoms that are highly debilitating and associated with a range of major physical health conditions (2, 3). PTSD disproportionately affects women over men (1, 4), and mechanisms for this sex difference have not yet been defined. We recently identified a single nucleotide polymorphism (SNP) in the gene coding for the pituitary adenylate cyclase-activating polypeptide (PACAP) receptor (*ADCYAP1R1*, adenylate cyclase activating polypeptide 1 receptor type 1) that predicts PTSD in women and not men (5–9). This SNP, rs2267735, is located on a canonical estrogen response element, indicating that estrogen levels may influence expression of the receptor. The *ADCYAP1R1* polymorphism has been shown to predict exaggerated arousal responses characteristic of PTSD in autonomic psychophysiology (5, 10), but no study has yet examined the extent to which this polymorphism influences fear responses in the human brain. The current study tests the hypothesis that *ADCYAP1R1* polymorphism influences brain regions that underlie emotional arousal, using functional MRI (fMRI) in a sample of women who have experienced civilian trauma.

PTSD symptom clusters include hyperarousal, reexperiencing, avoidance, and numbing (11). Recently, evidence has accumulated to support the idea that hyperarousal is predictive of PTSD after trauma, whereas other symptoms are products of the disorder (12). In particular, pretrauma reactivity of the amygdala—a brain region responsible for coordinating and maintaining multiple components of emotional arousal (13)—appears to be a predisposing risk factor for the maintenance of PTSD symptoms (14–16). Additional brain regions implicated in the pathophysiology of PTSD include the ventromedial and dorsolateral

prefrontal cortex, the anterior cingulate cortex, the hippocampus, and the insula (17, 18), each of which plays a role in regulating aspects of the emotional response.

Genetic profiles appear to be the initiating predictor of vulnerability to psychiatric disorders. In the case of PTSD, however, environmental factors play a similarly critical role, with a major example being the specific traumatic experience that is a necessary component of the disorder. The combined influences of genetics and the environment are also apparent in twin studies indicating that genetic factors account for 30–70% of PTSD risk, with higher estimates for women than men (19, 20), and the remaining variance is attributable to experience. Such interacting genetic and environmental influences create complex symptom profiles that can vary greatly from individual to individual (19, 21), introducing wide error margins in predicting initial vulnerability, progress of symptoms over time, or effective therapeutic courses for the individual.

Relative to genetic predictors or specific experiences, neurobiological phenotypes may provide greater power to predict psychopathology, as brain structure and function reflect an aggregate of genetic and environmental factors. Identifying such intermediate phenotypes, whose action falls between risk factors and psychiatric outcomes, will be critical to our ability to predict and understand disorders such as PTSD, helping to link multiple levels of research from proteins and cellular mechanisms to patient outcomes (21). Here we investigated the effects of an *ADCYAP1R1* polymorphism on amygdala and hippocampal function, in a

Significance

Higher circulating pituitary adenylate cyclase-activating polypeptide (PACAP) and a polymorphism in its receptor gene *ADCYAP1R1*, adenylate cyclase activating polypeptide 1 receptor type 1, have recently been linked with posttraumatic stress disorder (PTSD) in women and not men. The current study examined the influence of *ADCYAP1R1* genotype on brain function among traumatized women. In individuals with the risk genotype, the amygdala showed greater reactivity to threat stimuli and decreased functional connectivity with the hippocampus. *ADCYAP1R1* genotype had larger effects than PTSD diagnosis, suggesting that amygdala reactivity is an intermediate phenotype for anxiety-related psychopathology. Amygdala reactivity has been identified as a possible predisposing risk factor for PTSD, and the current findings indicate a possible genetic mechanism. Findings also point to a neurobiological explanation for increased PTSD prevalence in women.

Author contributions: J.S.S., L.M.A., N.F., B.B., T.J., and K.J.R. designed research; J.S.S., L.M.A., N.F., E.R., R.D., and E.M.G. performed research; J.S.S., D.A.G., and T.D.E. analyzed data; and J.S.S., L.M.A., N.F., D.A.G., B.B., S.D.N., E.R., T.D.E., R.D., E.M.G., T.J., and K.J.R. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

¹To whom correspondence should be addressed. E-mail: jennifer.stevens@emory.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1318954111/-DCSupplemental.

sample of adult women who have experienced moderate to high levels of civilian trauma. To disentangle genetic effects from the complex effects of PTSD and other psychiatric symptoms, genotype groups were matched for childhood and adult trauma levels and PTSD and depression symptom severity.

Our primary hypotheses focused on the amygdala because of its role in regulating emotional arousal and as a possible predisposing factor in PTSD. We also examined the hippocampus, another brain region that plays a central role in PTSD (22), where PACAP binding sites are particularly dense (23) and where PACAP has been shown to act in a neuroprotective or neurotrophic manner (24–26) and may facilitate synaptic plasticity (25, 27). We predicted that individuals with the risk polymorphism would show exaggerated amygdala responses to threat stimuli and decreased functional connectivity between the amygdala and the hippocampus and medial prefrontal cortex, regions that regulate amygdala reactivity (28, 29).

Results

Twenty-two participants with the *CC* genotype formed the risk group, and 27 participants with *GC* or *GG* genotypes formed the nonrisk group. Participants in the risk (*CC*) group did not differ from participants in the nonrisk (*GC/GG*) group in age, education, income, amount of trauma experienced, PTSD symptoms, depression symptoms, trait anxiety, or state anxiety immediately before the scan, as shown in Table 1.

fMRI Responses to Fearful Face Stimuli. All analyses were performed using the contrast of fearful > neutral face stimuli. In the amygdala region of interest (ROI), the risk group showed significantly greater activation than the nonrisk group (Fig. 1A; $p_{\text{corr}} < 0.05$). When each group was examined separately, the risk group showed bilateral amygdala activation ($p_{\text{corr}} < 0.05$; left: $Z = 3.52$, $k = 75$, $x, y, z = -32, -8, -16$; right: $Z = 3.12$, $k = 62$, $x, y, z = 24, -4, -28$), whereas the nonrisk group showed no significant amygdala activation. In the hippocampus ROI, the risk group showed significantly greater activation than the nonrisk group (Fig. 1B; $p_{\text{corr}} < 0.05$). When groups were examined separately, the risk group showed significant hippocampal activation bilaterally

($p_{\text{corr}} < 0.05$; left: $Z = 3.23$, $k = 148$, $x, y, z = -24, -12, -12$; right: $Z = 2.56$, $k = 136$, $x, y, z = 36, -8, -20$), whereas the nonrisk group showed no significant cluster of activation.

Similar results were obtained when ROI analyses were repeated using an additive model, examining voxels in which activation correlated linearly with the number of risk alleles (zero, one, or two C-alleles). Twenty-two participants had two risk alleles, 21 participants had one risk allele, and 6 participants had zero. The number of risk alleles correlated positively with activation in the amygdala bilaterally ($p_{\text{corr}} < 0.05$; left: $Z = 3.12$, $k = 62$, $x, y, z = -28, -8, -12$; right: $Z = 2.67$, $k = 40$, $x, y, z = 28, 0, -16$) and in the hippocampus bilaterally ($p_{\text{corr}} < 0.05$; left: $Z = 2.79$, $k = 112$, $x, y, z = -16, -36, -8$; right: $Z = 2.29$, $k = 53$, $x, y, z = 24, -36, -8$).

We then examined the effect sizes for group differences in amygdala activation associated with *ADCYAP1R1* genotype versus PTSD diagnosis as assessed by the MPSS. Cohen's d was calculated using an amygdala activation score extracted from each participant, using the mean contrast value across all voxels in the bilateral amygdala ROI for the fearful > neutral contrast. To examine PTSD effects on amygdala activation, we used a previously reported subset of the current sample that included 20 PTSD and 20 control participants matched for trauma experience (30). These participants completed the same experimental task used in the current study, and the PTSD effect on amygdala activation was $d = 0.20$. In the current study, the *ADCYAP1R1* effect was $d = 0.62$. Note that the use of a large anatomically defined ROI created a conservative estimate of effect size.

Table S1 and Fig. S1 show the results of exploratory whole-brain analyses, characterizing effects of *ADCYAP1R1* polymorphism on regions outside of the amygdala and hippocampus. Relative to the nonrisk group, the risk group showed increased responses in a large cluster with peaks in subgenual cingulate cortex and left amygdala, which also extended into the left caudate. A second large cluster contained peaks in left parahippocampal cortex and cerebellar regions and extended into the left thalamus. Notably, these clusters overlapped the left amygdala and left hippocampus ROIs. The nonrisk group did not show significantly increased activation in any region, relative to the risk

Table 1. Group characteristics

Demographic variable	<i>CC</i> ($n = 22$), M (SD)	<i>GC/GG</i> ($n = 27$), M (SD)	t
Age	37.6 (11.5)	39.6 (13.2)	-0.6
No. different traumas (TEI)	5.7 (3.0)	6.8 (7.2)	-0.7
Childhood trauma (CTQ)	44.5 (19.1)	42.8 (15.1)	0.3
Emotional abuse	8.8 (4.0)	9.3 (3.7)	-0.5
Physical abuse	7.9 (3.1)	7.7 (3.7)	0.2
Sexual abuse	10.3 (6.4)	9.9 (6.0)	0.2
PTSD symptoms (PSS)	14.6 (11.7)	16.3 (12.5)	-0.5
Intrusive	3.7 (3.3)	3.9 (3.7)	-0.2
Avoidance/numbing	5.5 (5.3)	7.0 (5.5)	-0.9
Hyperarousal	5.4 (4.4)	5.4 (4.2)	0.0
Depression symptoms (BDI)	12.4 (10.3)	13.7 (11.7)	-0.4
State anxiety (STAI)	34.1(10.4)	33.6(10.6)	0.2
Trait anxiety (STAI)	39.2(10.1)	36.1(9.3)	1.1
Years education, %			Mann-Whitney U
<12th grade	9.5	7.4	-1.8
12th grade/high school graduate	52.4	18.5	
General Educational Development	19.0	48.1	
Some college/technical school	9.5	11.1	
College/tech school graduate	9.5	14.8	
Monthly income			
\$0–249	20.0	18.5	
\$250–499	10.0	11.1	
\$500–999	30.0	40.7	
\$1,000–1,999	35.0	11.1	
\$2,000+	5.0	18.5	

risk genotype. These findings are consistent with previous evidence that *ADCYAP1R1* influences the startle reflex, an amygdala-modulated physiological response, producing exaggerated fear responses and a deficit in safety signal learning (5, 10). The risk genotype appears to be associated with decreased expression of the PACAP receptor because females with the risk polymorphism showed less *ADCYAP1R1* mRNA than those who did not have the risk polymorphism (5). The current findings point to one possible risk pathway for PTSD, in which the risk genotype predisposes individuals to increased fear reactivity through up-regulated amygdala responses related to decreased expression of the PACAP receptor.

We did not replicate our previous findings showing an association between *ADCYAP1R1* and PTSD symptoms. The small effect sizes typically observed for associations between genetic polymorphisms and psychiatric pathology require large sample sizes to reliably detect an effect (34). Here we took the complementary approach of balancing the genotype groups for PTSD symptoms and observed a significant link between *ADCYAP1R1* and amygdala function. In addition, we found that the effect size for *ADCYAP1R1* genotype was larger than the effect size for PTSD in a previous study using a similar sample (30). Taken together, the findings support the idea that tracing individual differences from genes through neural circuits that underlie pathological symptoms can provide a powerful approach to investigating complex psychiatric disease. With larger sample sizes, future research should focus on linking all three levels of analysis (genes, neural systems, and symptoms) to address questions about the extent to which neural activation and connectivity might add additional predictive value, above and beyond genotype, in predicting PTSD.

We also observed that *ADCYAP1R1* genotype influenced the hippocampal response to fearful stimuli. The hippocampus is involved in supporting fear expression and the inhibitory memory traces that form during fear extinction (28, 35) and is less activated in PTSD than control participants during extinction recall (36). Synaptic plasticity in the hippocampus is also critical to the formation and retrieval of conscious memories of personal experiences [episodic memory (37)]. Changes in hippocampal function are often observed in anxiety disorders, with several studies showing increased hippocampal activation in PTSD (38–41), hypothesized to reflect additional memory encoding or retrieval processes (38, 40). The hippocampus is particularly sensitive to stress hormones, which can impair plasticity (42, 43); significant early life stress, often in the form of childhood maltreatment, has been closely linked to reductions in hippocampal plasticity (44). Individuals with posttraumatic stress symptoms often experience changes in declarative memory function, such as intrusive recall of trauma (45), and a general impairment in remembering specific details (46). In the current study, the risk and nonrisk groups experienced equivalent levels of environmental stressors in the form of child and adult trauma, but hippocampal reactivity was increased only in the risk group. This finding raises the possibility that the *ADCYAP1R1* risk polymorphism increases hippocampal vulnerability to the harmful effects of stress. This question would be best addressed by a prospective study examining genotype and hippocampal structure and function before and after stress. It is also notable that although the risk group showed heightened reactivity to fearful stimuli in the amygdala and hippocampus, the nonrisk group showed very little reactivity in either region. This contrasts with findings in healthy individuals showing robust amygdala and hippocampal responses to fearful vs. neutral face stimuli (47). This discrepancy may be explained by the idea of genetic resiliency. The current findings may point to a particularly resilient group among individuals with the “nonrisk” (G) allele. Resilient individuals may show less reactivity or may adapt more quickly to threat stimuli. Future studies should directly address this possibility.

The risk polymorphism was also associated with decreased functional connectivity between the amygdala and hippocampus.

Interaction between these regions has been linked with multiple functions: pathways from amygdala to hippocampus are important for the modulatory effects of emotion on declarative memory encoding (48), whereas pathways from hippocampus to amygdala are necessary for contextual modulation of fear conditioning (49, 50). These are both cognitive mechanisms implicated in the maintenance of PTSD (38, 51). The *ADCYAP1R1* polymorphism did not influence interactions between the amygdala and ventromedial prefrontal cortex, despite the fact that dysregulated amygdala–prefrontal circuits are central neural features of PTSD (52, 53). Exploratory analyses showed the risk polymorphism was associated with decreased connectivity between the amygdala and a very anterior region of the medial prefrontal cortex, in BA 10, as well as other regions that may facilitate contextual modulation of amygdala activity including lateral temporal cortex and left caudate. However, *ADCYAP1R1* did not influence connectivity between the amygdala and several brain regions typically implicated in the pathophysiology of PTSD such as the anterior cingulate cortex and insula, suggesting that individual differences in the PACAP system may not explain all of the neural system changes associated with the disorder. It is important to note that the analyses used in the current paper cannot inform questions relating to the direction of interactions (e.g., whether one region influences the other).

Rodent studies of PACAP show that it has neurotrophic effects (24, 26) and may facilitate synaptic plasticity (25, 27). Plasticity in the amygdala and hippocampus is crucial to multiple forms of memory, playing a central role in reinforcement learning and declarative memory. Relatively less mRNA expression of the PACAP receptor, previously associated with the risk polymorphism, may lead to a decrease in plasticity in the amygdala and hippocampus, creating a pattern of inflexible fear memories that are less sensitive to context and changing environmental circumstances. Such a pattern would be highly consistent with the symptoms of PTSD and may have important implications for current therapeutic approaches that rely on neural plasticity for symptom improvement, such as prolonged exposure therapy. Future studies that specifically measure effects of *ADCYAP1R1* genotype on neural plasticity are needed.

Several limitations of the current study must be acknowledged. Because the *ADCYAP1R1* SNP, rs2267735, is located within a putative estrogen response element, PACAP may influence neural structure and function in an estrogen-dependent manner. Here we did not measure estrogen levels or estrous cycling. Future studies should investigate how individual differences in *ADCYAP1R1* interact with varying estrogen levels in adults and over development. Furthermore, our findings indicate that the PACAP system produces individual differences in brain function in a sample of participants who had all experienced significant levels of trauma. Future investigation should also address the role of PACAP function in a nontraumatized sample and possible contributions to sex differences in healthy responses to threat stimuli (54). In addition, the current study does not allow us to determine whether enhanced amygdala and hippocampal responses are specific to threat stimuli or may be observed across a variety of emotionally arousing stimuli. In PTSD, enhanced reactivity to emotional stimuli appears to be specific to threatening stimuli (32). However, the *ADCYAP1R1* risk polymorphism may increase emotional reactivity more broadly. Future research is needed to determine whether the genotype effects are specific to threat stimuli. Finally, the current study cannot disentangle whether the results represent direct effects of the polymorphism on expression of the PACAP receptor or whether they are linked to differential gene methylation or differential interaction with estrogen because these have been previously associated with PTSD in a similar cohort (5).

Conclusions

The current study demonstrated that a polymorphism in the PACAP receptor gene *ADCYAP1R1* influences neural responses to threat stimuli. The risk polymorphism, previously shown to predict PTSD in women, was associated with exaggerated amygdala

and hippocampal reactivity. This study highlights the utility of neuroimaging methods in characterizing intermediate phenotypes for PTSD and other complex psychiatric disorders. Polymorphisms in the PACAP receptor gene and amygdala and hippocampal phenotypes provide biomarkers that indicate individuals who may be more or less resilient to pathological stress symptoms following trauma and indicate a neurobiological pathway that may contribute to the greater prevalence of PTSD in women than men. Findings may also point to potential new treatment avenues, through enhancing the function of PACAP receptor-mediated pathways.

Materials and Methods

Participants. Forty-nine African-American women ages 18–62 were recruited through an ongoing study of risk factors for PTSD. MRI data from a subset of this sample have been reported elsewhere in a study of fMRI networks associated with PTSD (30). Because *ADCYAP1R1* has been linked to PTSD in women and not men (5, 7), the current investigation examined only women. Individuals who reported African-American race/ethnicity were recruited, to reduce effects of genetic admixture. Participants were approached in the general medical clinics of a large publicly funded hospital that serves economically disadvantaged individuals. High rates of childhood and adult trauma have previously been reported within this patient population (55, 56). Individuals reporting neurological disorder, current psychotropic medication, a history of bipolar disorder, schizophrenia or any current psychoses, or metal clips or implants were excluded. Participants had normal or corrected-to-normal vision. Urine tests for pregnancy and illegal drug use (cocaine, marijuana, opiates, amphetamines, and methamphetamines) were conducted 24 h before the MRI scan, and individuals who showed positive results were excluded. Participants received monetary compensation for their time. All participants provided written informed consent before participating. The institutional review board of Emory University approved the study procedures, and testing took place at Grady Memorial Hospital and the Biomedical Imaging Technology Center at Emory University Hospital.

Psychological Assessment. The Modified PTSD Symptom Scale (PSS) (57) was used to assess PTSD symptoms, and the Traumatic Events Inventory (TEI) was used to assess types and severity of trauma experience. Childhood trauma was assessed using the Childhood Trauma Questionnaire (CTQ) (58, 59). Anxiety levels were assessed using the State Trait Anxiety Inventory (STAI) (60) and depression symptoms were indexed using the Beck Depression Inventory (BDI) (61). These measures have been used in our previous studies with this population (5, 34). Trauma was assessed using the TEI and CTQ during recruitment, and the additional psychological measures were administered during a laboratory visit 1 d before the MRI scan. All participants had experienced at least one trauma.

Genotyping and Analysis. Saliva was collected in Oragene tubes (DNA Genotek Inc.). A 500- μ L aliquot was used for extraction using the DNAAdvance extraction kit (Beckman Coulter Genomics). The resulting salivary DNA was quantified using gel electrophoresis and Quantity One software (Bio-Rad) or NanoDrop2000 (Thermo Fisher Scientific Inc.) and then normalized to 10 ng/ μ L. A total amount of 15 ng of DNA per reaction was transferred to the genotyping plates and then dried down before the reactions. Reactions were performed on the Taqman ViiA7 Real-Time PCR system using Taqman SNP Genotyping Assay Mix with Taqman GTXpress Master Mix (Life Technologies Inc.) and on the Sequenom SNP genotyping platform using iPLEX gold reagents and the MassARRAY system (Sequenom Inc.). The *ADCYAP1R1* variant rs2267735 passed Hardy–Weinberg Equilibrium with a P value of 0.90. The call rates on both platforms were greater than 95%. Quality control measures, including within plate duplicates and nontemplate controls, were used. In addition, 25% of the samples were duplicated on both platforms. There were no discordant calls.

Following the group comparison strategy in ref. 5, participants were categorized into two groups on the basis of genotype: individuals with two copies of the C allele, previously associated with risk for PTSD, were assigned to the risk group (CC), whereas individuals with one or zero copies of the C allele (GC/GG) were assigned to the nonrisk group. To verify genotype effects, a secondary analysis was conducted using an additive model (using the number of risk alleles: zero, one, or two C-alleles).

fMRI Threat-Processing Task. The task involved passive viewing of fearful and neutral face stimuli. This task has been shown to engage threat-processing networks in previous studies (32, 33, 62), and the specific procedures are described elsewhere (30). Fearful and neutral face stimuli (63) were

presented in a block design. Trials included a face stimulus presented for 500 ms, followed by a 500-ms presentation of a fixation cross. Participants were instructed to pay attention to the faces and did not make any behavioral response, to minimize motion artifacts and neural activation unrelated to processing the visual stimulus.

Brain Imaging Acquisition and Analysis. Brain imaging data were acquired on a Siemens 3.0 Tesla Magnetom Trio (Siemens) using a 12-channel head coil. Functional images were acquired using the Z-SAGA pulse sequence (64) to minimize signal loss due to susceptibility artifacts. Volumes were acquired axially, parallel to the anterior–posterior commissure line [30 slices, $3.44 \times 3.44 \times 4$ mm, repetition time (TR) = 3,000 ms, echo time (TE) 1 = 30 ms, TE2 = 67 ms, flip angle = 90°]. Structural images were acquired using a gradient-echo, T1-weighted pulse sequence (176 slices, $1 \times 1 \times 1$ mm, TR = 2,600 ms, TE = 3.02 ms, flip angle = 8°).

Functional images were corrected for slice timing and realigned using Analysis of Functional Neuroimages (AFNI) software. The matrix to coregister the echo-planar images (EPI) to the anatomical image was calculated using Functional MRI of the Brain (FMRIB) Software Library (FSL). The anatomical image was registered and normalized into standard Montreal Neurological Institute space using FSL, and the resulting matrix was combined with the coregistration matrix and applied to the functional images. Functional images were then smoothed with an 8-mm Gaussian kernel.

For each participant, first-level general linear models were estimated using Statistical Parametric Mapping software (SPM5) (65). Evoked hemodynamic responses for blocks of fearful and neutral stimuli were modeled with a boxcar function representing the onset and 8,000 ms duration of the block, convolved with a canonical hemodynamic response function. Motion parameters were included as covariates. Statistical contrasts between conditions (e.g., fearful vs. neutral) were assessed using linear contrasts. Contrast images representing the linear comparison of beta values for the fearful versus neutral conditions were entered into group-level random effects analysis to identify clusters of significant activation. To address the a priori prediction that the *ADCYAP1R1* polymorphism would influence amygdala and hippocampus activation, we conducted ROI analyses, with bilateral masks defined anatomically using the SPM Anatomy Toolbox (66). Exploratory whole-brain analyses were also conducted to examine the effect of *ADCYAP1R1* genotype across a broader range of regions involved in processing emotional or social stimuli.

Task-based functional connectivity analyses were conducted using the CONN toolbox (67). Seed regions were defined anatomically using the mean time course across voxels within the right and left amygdala, and covariance with these regions was examined voxel-wise across the whole brain. Individual participants' motion parameters were modeled as nuisance covariates. Spontaneous, non-task-related covariance between regions was controlled by examining statistical contrasts of connectivity for fearful relative to neutral face stimuli, such that results included only regions that showed significantly increased connectivity with the amygdala for fearful relative to neutral faces. The resulting contrast images for individual participants were used group-level analyses comparing participants with and without the risk allele. Results were restricted to hippocampal and prefrontal ROIs. The hippocampal ROI was defined using the same anatomical mask image used in the analysis of regional activation. The prefrontal ROI included anterior cingulate and medial prefrontal cortex regions that are involved in emotion regulation (68–70) and show deficits in regulating amygdala reactivity in PTSD (30, 52). The ROI included BA 24, 25, and 32, restricted to anterior aspects of the prefrontal cortex using y coordinates greater than +0.

Regional activation and functional connectivity analyses were conducted using a combined height–extent threshold to correct for multiple comparisons, using AlphaSim within the REST toolbox (71). For whole-brain analyses, the corrected height–extent threshold was calculated for voxels within a gray matter mask based on the International Consortium for Brain Mapping (ICBM) 152-subject template. A cluster-forming threshold of $P < 0.01$ was used and when combined with a cluster size of $k = 19$ resulted in a corrected probability of $P < 0.043$, (voxel-wise probability $P < 0.0006$). For ROI analyses, a cluster-forming threshold of $P < 0.05$ was used. Within the amygdala ROI, a cluster size of $k = 11$ resulted in a corrected probability of $P < 0.049$ (voxel-wise probability $P < 0.005$). Within the hippocampus ROI, a cluster size of $k = 17$ resulted in a corrected probability of $P < 0.045$ (voxel-wise probability $P < 0.003$). Within the prefrontal ROI, a cluster size of $k = 32$ resulted in a corrected probability of $P < 0.042$ (voxel-wise probability $P < 0.002$).

ACKNOWLEDGMENTS. We appreciate the technical support of all of the staff and volunteers of the Grady Trauma Project, in particular Allen W. Graham and Angelo Brown. We thank the participants of the Grady Trauma Project for their time and effort. This work was primarily supported by the National Institutes of Mental Health (MH096764 and MH071537 to K.J.R.). Support was

also received from Emory and Grady Memorial Hospital General Clinical Research Center, National Institutes of Health (NIH) National Centers for Research Resources (M01RR00039), and the National Center for Advancing

Translational Sciences of the NIH (UL1TR000454). The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs or the United States government.

- Kessler RC, et al. (2005) Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 62(6):593–602.
- Pacella ML, Hruska B, Delahanty DL (2013) The physical health consequences of PTSD and PTSD symptoms: A meta-analytic review. *J Anxiety Disord* 27(1):33–46.
- McDevitt-Murphy ME, et al. (2010) PTSD symptoms, hazardous drinking, and health functioning among U.S. OEF and OIF veterans presenting to primary care. *J Trauma Stress* 23(1):108–111.
- True WR, et al. (1993) A twin study of genetic and environmental contributions to liability for posttraumatic stress symptoms. *Arch Gen Psychiatry* 50(4):257–264.
- Ressler KJ, et al. (2011) Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. *Nature* 470(7335):492–497.
- Dias BG, Ressler KJ (2013) PACAP and the PAC1 receptor in post-traumatic stress disorder. *Neuropsychopharmacology* 38(1):245–246.
- Almli LM, et al. (2013) ADCYAP1R1 genotype associates with post-traumatic stress symptoms in highly traumatized African-American females. *Am J Med Genet B Neuropsychiatr Genet* 162B(3):262–272.
- Uddin M, et al. (2012) ADCYAP1R1 genotype, posttraumatic stress disorder, and depression among women exposed to childhood maltreatment. *Depress Anxiety* 30(3):251–258.
- Wang L, et al. (2013) PAC1 receptor (ADCYAP1R1) genotype is associated with PTSD's emotional numbing symptoms in Chinese earthquake survivors. *J Affect Disord* 150(1):156–159.
- Jovanovic T, et al. (2013) PAC1 receptor (ADCYAP1R1) genotype is associated with dark-enhanced startle in children. *Mol Psychiatry* 18(7):742–743.
- American Psychiatric Association (2000) *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR)* (Am Psychiatr Assoc, Washington, DC), 4th Ed.
- Admon R, Milad MR, Hendlar T (2013) A causal model of post-traumatic stress disorder: disentangling predisposed from acquired neural abnormalities. *Trends Cogn Sci* 17(7):337–347.
- LeDoux JE (2000) Emotion circuits in the brain. *Annu Rev Neurosci* 23(1):155–184.
- Dannlowski U, et al. (2013) Childhood maltreatment is associated with an automatic negative emotion processing bias in the amygdala. *Hum Brain Mapp* 34(11):2899–2909.
- Dannlowski U, et al. (2012) Limbic scars: Long-term consequences of childhood maltreatment revealed by functional and structural magnetic resonance imaging. *Biol Psychiatry* 71(4):286–293.
- Admon R, et al. (2009) Human vulnerability to stress depends on amygdala's predisposition and hippocampal plasticity. *Proc Natl Acad Sci USA* 106(33):14120–14125.
- Liberzon I, Sripada CS (2008) The functional neuroanatomy of PTSD: A critical review. *Prog Brain Res* 167:151–169.
- Hayes JP, VanElzakker MB, Shin LM (2012) Emotion and cognition interactions in PTSD: A review of neurocognitive and neuroimaging studies. *Front Integr Neurosci* 6:89.
- Stein MB, Jang KL, Taylor S, Vernon PA, Livesley WJ (2002) Genetic and environmental influences on trauma exposure and posttraumatic stress disorder symptoms: A twin study. *Am J Psychiatry* 159(10):1675–1681.
- Sartor CE, et al. (2011) Common genetic and environmental contributions to post-traumatic stress disorder and alcohol dependence in young women. *Psychol Med* 41(7):1497–1505.
- Caspi A, Moffitt TE (2006) Gene-environment interactions in psychiatry: Joining forces with neuroscience. *Nat Rev Neurosci* 7(7):583–590.
- Shin LM, Rauch SL, Pitman RK (2006) Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. *Ann N Y Acad Sci* 1071:67–79.
- Masuo Y, et al. (1991) Autoradiographic distribution of pituitary adenylate cyclase activating polypeptide (PACAP) binding sites in the rat brain. *Neurosci Lett* 126(2):103–106.
- Mercer A, et al. (2004) PACAP promotes neural stem cell proliferation in adult mouse brain. *J Neurosci Res* 76(2):205–215.
- Yang K, Lei G, Jackson MF, Macdonald JF (2010) The involvement of PACAP/VIP system in the synaptic transmission in the hippocampus. *J Mol Neurosci* 42(3):319–326.
- Lindholm DAN, Skoglösa Y, Takei N (1998) Developmental regulation of pituitary adenylate cyclase activating polypeptide (PACAP) and its receptor 1 in rat brain: Function of PACAP as a neurotrophic factor. *Ann N Y Acad Sci* 865(1):189–196.
- Masuo Y, Matsumoto Y, Tokito F, Tsuda M, Fujino M (1993) Effects of vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase activating polypeptide (PACAP) on the spontaneous release of acetylcholine from the rat hippocampus by brain microdialysis. *Brain Res* 611(2):207–215.
- Milad MR, et al. (2007) Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. *Biol Psychiatry* 62(5):446–454.
- Pezawas L, et al. (2005) 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: A genetic susceptibility mechanism for depression. *Nat Neurosci* 8(6):828–834.
- Stevens JS, et al. (2013) Disrupted amygdala-prefrontal functional connectivity in civilian women with posttraumatic stress disorder. *J Psychiatr Res* 47(10):1469–1478.
- Sabatinelli D, et al. (2011) Emotional perception: Meta-analyses of face and natural scene processing. *Neuroimage* 54(3):2524–2533.
- Rauch SL, et al. (2000) Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: A functional MRI study. *Biol Psychiatry* 47(9):769–776.
- Shin LM, et al. (2005) A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. *Arch Gen Psychiatry* 62(3):273–281.
- Fani N, et al. (2013) FKBP5 and attention bias for threat: associations with hippocampal function and shape. *JAMA Psychiatry* 70(4):392–400.
- Sierra-Mercado D, Padilla-Coreano N, Quirk GJ (2011) Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. *Neuropsychopharmacology* 36(2):529–538.
- Milad MR, et al. (2009) Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biol Psychiatry* 66(12):1075–1082.
- Tulving E, Markowitsch HJ (1998) Episodic and declarative memory: Role of the hippocampus. *Hippocampus* 8(3):198–204.
- Thomas K, et al. (2013) Increased anterior cingulate cortex and hippocampus activation in complex PTSD during encoding of negative words. *Soc Cogn Affect Neurosci* 8(2):190–200.
- Werner NS, et al. (2009) Hippocampal function during associative learning in patients with posttraumatic stress disorder. *J Psychiatr Res* 43(3):309–318.
- Geuze E, et al. (2007) Altered pain processing in veterans with posttraumatic stress disorder. *Arch Gen Psychiatry* 64(1):76–85.
- Shin LM, et al. (2004) Hippocampal function in posttraumatic stress disorder. *Hippocampus* 14(3):292–300.
- McEwen BS (1999) Stress and hippocampal plasticity. *Annu Rev Neurosci* 22(1):105–122.
- de Kloet ER, Oitzl MS, Joels M (1999) Stress and cognition: Are corticosteroids good or bad guys? *Trends Neurosci* 22(10):422–426.
- McEwen BS (2012) Brain on stress: How the social environment gets under the skin. *Proc Natl Acad Sci USA* 109(Suppl 2):17180–17185.
- Ehlers A, Steil R (1995) Maintenance of intrusive memories in posttraumatic stress disorder: A cognitive approach. *Behav Cogn Psychother* 23(3):217–249.
- Williams JMG, et al. (2007) Autobiographical memory specificity and emotional disorder. *Psychol Bull* 133(1):122–148.
- Williams LM, et al. (2001) Arousal dissociates amygdala and hippocampal fear responses: Evidence from simultaneous fMRI and skin conductance recording. *Neuroimage* 14(5):1070–1079.
- McGaugh JL (2002) Memory consolidation and the amygdala: A systems perspective. *Trends Neurosci* 25(9):456–461.
- Alvarez RP, Biggs A, Chen G, Pine DS, Grillon C (2008) Contextual fear conditioning in humans: Cortical-hippocampal and amygdala contributions. *J Neurosci* 28(24):6211–6219.
- Orsini CA, Kim JH, Knapska E, Maren S (2011) Hippocampal and prefrontal projections to the basal amygdala mediate contextual regulation of fear after extinction. *J Neurosci* 31(47):17269–17277.
- Jovanovic T, Ressler KJ (2010) How the neurocircuitry and genetics of fear inhibition may inform our understanding of PTSD. *Am J Psychiatry* 167(6):648–662.
- Sripada RK, et al. (2012) Altered resting-state amygdala functional connectivity in men with posttraumatic stress disorder. *J Psychiatry Neurosci* 37(4):241–249.
- Etkin A, Wager TD (2007) Functional neuroimaging of anxiety: A meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Am J Psychiatry* 164(10):1476–1488.
- Stevens JS, Hamann S (2012) Sex differences in brain activation to emotional stimuli: A meta-analysis of neuroimaging studies. *Neuropsychologia* 50(7):1578–1593.
- Binder EB, et al. (2008) Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA* 299(11):1291–1305.
- Gillespie CF, et al. (2009) Trauma exposure and stress-related disorders in inner city primary care patients. *Gen Hosp Psychiatry* 31(6):505–514.
- Foa EB, Tolin DF (2000) Comparison of the PTSD symptom scale-interview version and the clinician-administered PTSD scale. *J Trauma Stress* 13(2):181–191.
- Bernstein DP, et al. (1994) Initial reliability and validity of a new retrospective measure of child abuse and neglect. *Am J Psychiatry* 151(8):1132–1136.
- Scher CD, Stein MB, Asmundson GJG, McCreary DR, Forde DR (2001) The childhood trauma questionnaire in a community sample: Psychometric properties and normative data. *J Trauma Stress* 14(4):843–857.
- Spielberger CD, Gorsuch RL, Lushene RE (1970) *The State-Trait Anxiety Inventory: Test Manual* (Consult Psychol Press, Palo Alto, CA).
- Beck AT, Steer RA, Carbin MG (1988) Psychometric properties of the Beck Depression Inventory: Twenty-five years of evaluation. *Clin Psychol Rev* 8(1):77–100.
- Breiter HC, et al. (1996) Response and habituation of the human amygdala during visual processing of facial expression. *Neuron* 17(5):875–887.
- Ekman P, Friesen WV (1976) *Pictures of Facial Affect* (Consult Psychol Press, Palo Alto, CA).
- Heberlein KA, Hu X (2004) Simultaneous acquisition of gradient-echo and asymmetric spin-echo for single-shot z-shim: Z-SAGA. *Magn Reson Med* 51(1):212–216.
- Holmes AP, Friston KJ (1998) Generalisability, random effects and population inference. *Neuroimage* 7:5754.
- Eickhoff SB, et al. (2005) A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *Neuroimage* 25(4):1325–1335.
- Whitfield-Gabrieli S, Nieto-Castanon A (2012) Conn: A functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connectivity* 2(3):125–141.
- Etkin A, Egner T, Kalisch R (2011) Emotional processing in anterior cingulate and medial prefrontal cortex. *Trends Cogn Sci* 15(2):85–93.
- Kim SH, Hamann S (2007) Neural correlates of positive and negative emotion regulation. *J Cogn Neurosci* 19(5):776–798.
- Milad MR, et al. (2007) A role for the human dorsal anterior cingulate cortex in fear expression. *Biol Psychiatry* 62(10):1191–1194.
- Song X-W, et al. (2011) REST: A toolkit for resting-state functional magnetic resonance imaging data processing. *PLoS ONE* 6(9):e25031.